specification. Addition of a monosaccharide or sulfate group is disclosed as occurring at the non-reducing end of an oligosaccharide, polysaccharide or carbohydrate on a biomolecule. A biomolecule is defined as a biologically significant molecule and includes carbohydrates and lipids. Synthesis of oligosaccharides is described in more detail at pages 49-54. Synthesis of glycolipids is also described in detail beginning at page 54, line 24 through page 56, line 3. This amendment adds no new matter.

Claim 53 was also amended to include a heterologous glycosyltransferase. Support for use of a heterologous enzyme is found at page 10 of the specification. A heterologous polypeptide is defined as one that originated from a source foreign to the particular host cell, or if from the same source, is modified from its original form. This amendment adds no new matter.

Claims 53 and 59 were amended to recite the term accessory enzyme. Support for use of the term accessory enzyme is found in the specification at, for example, page 11. Accessory enzyme is defined as an enzyme that catalyzes a reaction that forms a substrate for a glycosyltransferase. Examples given include an enzyme that catalyzes the formation of a nucleotide sugar used as a donor moiety by a glycosyltransferase, an enzyme used in formation of a nucleotide triphosphate, or enzyme used in generation of the sugar incorporated into the nucleotide sugar. Numerous specific examples of accessory enzymes are found throughout the application. This amendment adds no new matter.

New claim 72 is directed to use of the catalytic domain of a glyscosyltransferase. A catalytic domain is defined as a portion of an enzyme that is sufficient to catalyze an enzymatic reaction normally carried out by an enzyme. Specification at page 11. This amendment adds no new matter.

2. Rejection under U.S.C. §112, first paragraph: written description

Claims 53-59, 61, and 66-67 were rejected under 35 U.S.C. §112, first paragraph as allegedly containing subject matter which was not described in the specification as originally filed. In the Office Action the Examiner observed that the

purpose of the written description requirement is to convey to one skilled in the relevant art that the inventors had possession of the claimed invention as of the filing date.

The Examiner objected to the use of the phrases "enzymatic system for forming a nucleotide sugar" and "fusion protein" in the claims, alleging that nine representative species of sugar cycles were not sufficient to describe a genus for forming a nucleotide sugar, and further alleging that one disclosed fusion protein was not sufficient to describe a genus of fusion proteins comprising a catalytic domain from a glycosyltransferase and a catalytic domain from an accessory enzyme. Applicants assert that the species pointed to by the Examiner, in combination with the description provided in the specification and information available to those of skill in the art, adequately describe the claimed genera.

a. The specification provides adequate written description for accessory enzymes and enables their use as claimed.

The Examiner alleged that the claims are "directed to the use of any enzymatic system for forming a nucleotide sugar." Office Action at page 2. The Examiner also alleged that the specification provided only representative sugar nucleotide regeneration cycles found at Table 1, page 28 of the Specification. The Examiner further alleged there was no written description of any other enzymatic system for forming a nucleotide sugar and that the specification failed to describe any additional representative species of enzymatic systems for forming a nucleotide sugar.

Applicants have amended claim 53 and dependent claims to specify an "accessory enzyme" rather than an "enzymatic system" for forming a nucleotide sugar. To the extent that the rejection applies to the claims as amended, Applicants respectfully traverse.

Accessory enzyme is defined in the specification at page 11, lines 17-22 as an enzyme that catalyzes a reaction that forms a substrate for a glycosyltransferase. Examples given include an enzyme that catalyzes the formation of a nucleotide sugar used as a donor moiety by a glycosyltransferase, an enzyme used in formation of a

nucleotide triphosphate, or an enzyme used in generation of the sugar incorporated into the nucleotide sugar.

The claimed invention is a method of using accessory enzymes in combination with glycosyltransferases to form specific product saccharides. Accessory enzymes are well known to those of skill in the art and need not be described in detail. *Hybritech v. Monoclonal Antibodies*, 802 F.2d 1367, 1384 (Fed. Cir. 1986) (The description need only describe in detail that which is new or not conventional.)

The written description of a claimed genus can be satisfied through sufficient description of a representative number of species. *Regents of the University of California v. Eli Lilly*, 119 F.3d 1559, 1568 (Fed. Cir. 1997). A representative number of species are found in the nine disclosed sugar cycles, the specific accessory enzymes from prokaryotic and eukaryotic sources found in the specification, and in the knowledge of those of skill in the art.

The disclosed accessory enzymes are adequately described and enable the use of the claimed methods. The specification provides specific examples of at least 12 accessory enzymes for forming a nucleotide sugar. Specification at pages 29-34. Table 1 at page 28, also provides nine examples of cycles for forming nucleotide sugars, including the enzymes required to regenerate a nucleotide triphosphate from a nucleotide. Using a single enzyme as an example, at page 32 of the specification, ten UDP-Gal pyrophosphorylase enzymes with accession numbers are disclosed. Six are from prokaryotic or viral organisms: *Lactobacillus*, *E. coli*, *B. subtilis*, *Neisseria*, *Haemophilus*, and *Streptomyces*. Four are from eukaryotes: Rat, bakers yeast, mouse, and human. Similar analysis is done for the other enzymes disclosed throughout 6 pages of the specification. Thus, by providing detailed information about the identity of accessory enzymes in the specification combined with well-known characteristics of enzymatic pathways to synthesize nucleotide sugars, applicants have adequately described the invention.

Those of skill in the art are aware of the substrates for glycosyltransferases, the enzymatic pathways used to synthesize nucleotide sugars, the

enzymes used to form a nucleotide triphosphate, and the enzymes used in generation of the sugar incorporated into the nucleotide sugar. Descriptions of reactions catalyzed by glycosyltransferases and formation of nucleotide sugars are given, for example, in biochemical textbooks, as are enzymes used in formation of nucleoside triphosphates, and enzymes that synthesize sugar molecules. *See e.g.* Lehninger, *Principles of Biochemistry* (1984); Stryer, *Biochemistry* (1995); Zubay, *Biochemistry* (1986).

Given that the claimed accessory enzymes are well known to those of skill in the art and are further described in the specification, the written description provided in the specification has identified a representative number of accessory enzymes and adequately describes the genus of accessory enzymes. Thus, the claimed methods of use of accessory enzymes is also adequately described in the specification.

b. The specification provides adequate written description and enablement for methods to use fusion proteins as claimed.

The Examiner alleges the specification provides only one representative sample of a fusion protein encompassed by the claims, CMP-sialic acid/α-2,3sialyltransferase, and that no other representative species or structural characteristics are provided. Office Action at page 3. Applicants respectfully traverse and assert that the application adequately describes the characteristics of a representative number of fusion proteins useful in the claimed methods.

A fusion protein is defined as having more than one enzymatic activity that is involved in the synthesis of a desired oligosaccharide. The specification also states that a fusion protein can be composed of a catalytic domain of a glycosyltransferase joined in frame to a catalytic domain of an accessory enzyme. Specification at page 37, lines 4-19.

Catalytic domain is defined in the specification as a portion of an enzyme that is sufficient to carry out an enzymatic reaction normally carried out by the enzyme. Specification at page 11, lines 23-28.

Glycosyltransferase and accessory enzyme are also defined in the specification. Glycosyltransferase is defined as catalyzing the transfer of a sugar from a nucleotide sugar to an acceptor sugar to form a product saccharide. Specification at page 7, lines 19-21. The definition of accessory enzyme was discussed above.

Both accessory enzymes and glycosyltransferases are adequately described in the specification and will enable those of skill in the art to use fusion proteins comprising them in the claimed methods. The support for accessory enzymes was described previously. Glycosyltransferases are similarly well described in the specification. Eukaryotic glycosyltransferases are described from page 19, line 1 through page 21, line 15. Embodiments include fucosyltransferases, galactosyltransferases, sialyltransferases, glucosyltransferases, N-acetylgalactosaminyltransferases, and mannosyltransferases. References are cited for enzymes from many different organisms and some Accession Numbers are given. For example, galactosyltransferases are listed starting at page 19, line 25 through page 20, line14. Twelve different galactosyltransferases are disclosed from human, bovine, murine, and porcine sources, as well as an enzyme form the yeast *Schizosaccharomyces pombe*. α(1,3), α(1,2), and α(1,4) galactosyltransferases are disclosed, as is a ceramide galactosyltransferase. Similar disclosure is made for the other eukaryotic glycosyltransferases.

A number of prokaryotic glycosyltransferases are also disclosed starting at page 21, line 16 through page 22, line 25. Prokaryotic glycosyltransferases include enzymes involved in synthesis of LOS. Disclosed prokaryotic enzymes include galactosyltransferases, glucosyltransferases, N-acetylglucosaminyltransferases, fucosyltransferasese, and glycosyltranseferases involved in the synthesis of structures containing lacto-N-neotetraose. The enzymes come from a variety of prokaryotic sources, including *E. coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *S. enterica*, *Yersinia enterocolitica*, *Mycobacterium leprosum*, *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, and *N. meningitidis*. References are cited for each enzyme disclosed and some accession numbers are given.

Both accessory enzymes and glycosyltransferases are well known to those of skill in the art. In addition to the disclosure found in the specification, numerous reference materials are available that discuss accessory enzymes and glycosyltransferases, for example biochemical textbooks. *See e.g.* Lehninger, *Principles of Biochemistry* (1984); Stryer, *Biochemistry* (1995); Zubay, *Biochemistry* (1986). As discussed above, neither accessory enzyme, nor glycosyltransferases must be described in detail in the specification. *Hybritech*, 802 F.2d at 1384.

Those of skill in the art can easily identify appropriate combinations of accessory enzymes and glycosyltransferases for use in a fusion protein. For example, one of skill in the art would know to combine an accessory enzyme for formation of a nucleotide-**galactose** molecule with a **galactosyltransferase** to make a fusion protein according to the present invention.

The disclosure of catalytic domains of accessory enzymes and prokaryotic and eukaryotic glycosyltransferases, the specific embodiment of the fusion protein disclosed in the specification, and the knowledge of those of skill in the art provide a representative number of fusion proteins sufficient to adequately describe the claimed genus of fusion proteins. Thus, the claimed methods of use of fusion proteins are also adequately described and are also enabled.

In view of the above amendments and remarks, Applicants respectfully request that the rejections under 35 U.S.C. §112, first paragraph be withdrawn.

3. Rejections under U.S.C. §112, second paragraph

a. Omission of essential steps.

The Examiner rejected claims 53-71 under 35 U.S.C. §112, second paragraph as allegedly being incomplete for omitting essential steps. The Examiner cited isolating and recovering the produced product saccharide as an omitted essential step.

Applicants respectfully disagree that isolating and recovering the product saccharide is an essential step for the claimed method. In order to be rejected under 35

U.S.C. §112, second paragraph for omitting essential steps, a claim must omit matter disclosed to be essential to the invention **in the specification**.

The specification clearly states that the steps of isolating and recovering the product saccharide are not essential for the claimed method. At page 46, line 6-7 the specification states the following: "The products formed by the above processes (*e.g.* product saccharides) can be used without purification." Thus, Applicants have not disclosed the steps of isolating and recovering the product saccharide as essential to the claimed method. The rejection for omission of essential steps was improper and should be withdrawn.

b. Indefiniteness

Claims 53-59, 61, and 65-71 were rejected under 35 U.S.C. §112, second paragraph as allegedly being indefinite. For claim 53, the Examiner alleged specific enzymes composing an "enzymatic system" and specific "nucleotide sugar(s)" are not known and are not described by the specification. For claim 59, the Examiner alleged that the phrase " the enzymatic system for forming a nucleotide sugar comprises an enzyme" was indefinite because the specific enzyme was not known and was not recited in the claim. Office Action at pages 3-4.

Applicants have amended the claims to specify an "accessory enzyme" rather than an "enzymatic system" for forming a nucleotide sugar. To the extent the rejections apply to the claims as amended, applicants respectfully traverse.

Applicants assert that one of ordinary skill in the art would understand the claimed invention in light of the specification. "[35 U.S.C.] §112, second paragraph, requires a determination of whether those skilled in the art would understand what is claimed in light of the specification." *Orthokinetics v. Safety Travel Chairs Inc.*, 1 USPQ2d 1081 (Fed. Cir. 1986).

As stated above, accessory enzyme is defined in the specification at page 11, lines 17-22 as an enzyme that catalyzes a reaction that forms a substrate for a glycosyltransferase. Three examples of accessory enzymes are given: (A) an enzyme

that catalyzes the formation of a nucleotide sugar used as a donor moiety by a glycosyltransferase, (B) an enzyme used in formation of a nucleotide triphosphate, and (C) an enzyme used in generation of the sugar incorporated into the nucleotide sugar.

The Examiner alleged the term "nucleotide sugar" was not defined and the specific nucleotide sugar was not known. A nucleotide sugar formed by an accessory enzyme is an activated nucleotide sugar. Specification at page 9, lines 3-7. Activated nucleotide sugars are described as generally consisting of uridine and guanosine diphosphates and cytidine monophosphate derivatives of sugars, in which the nucleoside diphosphate or monophosphate serves as a leaving group. It is noted that bacterial, plant and fungal systems sometimes use other activated sugars. Thus other nucleotide sugars are encompassed by the present invention, so long as they are used to synthesize oligosaccharides. Those of skill in the art would understand the definition of nucleotide sugar found in the specification.

In view of the above amendments and remarks, Applicants respectfully request that the rejections under 35 U.S.C. §112, second paragraph be withdrawn.

4. Rejection under U.S.C. §102(a)

Claims 53, 54, 56-62, 66, 69, and 71 were rejected under U.S.C. §102(a) as allegedly being anticipated by Gilbert *et al.* The Examiner states that Gilbert *et al.* teach a fusion protein that has both CMP-Neu5Ac synthetase and α-2,3-sialyltransferase activities. Using genes cloned from *Neisseria meningitidis*, the fusion protein was expressed in *E. coli* and used to make sialylated oligosaccharides. The Examiner alleges these teachings anticipate the claimed invention. Office action at page 4.

To anticipate a claim, the reference must teach every element of the claim. "A claim is anticipated only if each and every element as set forth in the claim is found...in a single prior art reference." *Verdegaal Bros. v. Union Oil of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). Thus, in order to anticipate, Gilbert *et al.* must contain every element of the claims at issue.

Gilbert *et al.* does not anticipate the claims at issue. The claimed invention is a method of producing a product saccharide within a microorganism or plant cell. The microorganism or plant cell comprises an accessory enzyme for forming a nucleotide sugar and a recombinant glycosyltransferase.

In contrast, Gilbert *et al.* describes an **acellular** method of forming an oligosaccharide. The method uses a CMP-Neu5Ac synthetase/α-2,3-sialyltransferase fusion protein that is at least 90% pure.

Using the purification methods of Gilbert *et al.*, cells are destroyed and most cellular material is discarded before production of the product saccharide begins. The purification methods include cell disruption using an Avestin C5 Emulsiflex cell disrupter, PEG precipitation and separation of soluble and insoluble material, membrane solubilization with Triton X-100, and chromatography on a HiTrap Chelating column charged with Ni. Cells are not contacted with the substrate sugar as required by the claims. Thus, Gilbert *et al.* does not disclose synthesis of product saccharides using cells.

Because Gilbert *et al.* does not include the element of producing a product oligosaccharide using a cellular system, the reference does not contain all of the elements of the claimed invention and does not anticipate the invention under 35 U.S.C. §102(a). Applicants therefore respectfully request that the rejection under 35 U.S.C. §102(a) be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

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APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE

53. (Once amended) A method of producing a product saccharide, wherein the product saccharide is an oligosaccharide or glycolipid, the method comprising contacting a microorganism or plant cell with an acceptor saccharide, wherein the cell comprises:

a) an [enzymatic system] accessory enzyme for forming a nucleotide sugar; and

b) a [recombinant] heterologous glycosyltransferase which catalyzes the transfer of a sugar from the nucleotide sugar to the acceptor saccharide to produce the product saccharide.

54. (Cancel) The method of claim 53, wherein the glycosyltransferase is encoded by a heterologous gene.

59. (Once amended) The method of claim 53, wherein the [enzymatic system] accessory enzyme for forming a nucleotide sugar comprises an enzyme that is encoded by a heterologous gene.

72. (New) The method of claim 53, wherein the glycosyltransferase consists essentially of a catalytic domain of the glycosyltransferase.

APPENDIX B

PENDING AND AMENDED CLAIMS

53. (Once amended) A method of producing a product saccharide, wherein the product saccharide is an oligosaccharide or glycolipid, the method comprising contacting a microorganism or plant cell with an acceptor saccharide, wherein the cell comprises:

a) an accessory enzyme for forming a nucleotide sugar; and

b) a heterologous glycosyltransferase which catalyzes the transfer of a sugar from the nucleotide sugar to the acceptor saccharide to produce the product saccharide.

55. (As filed) The method of claim 53, wherein the glycosyltransferase is encoded by a gene that is endogenous to the cell and is produced by the cell at an elevated level compared to a wild-type cell.

56. (As filed) The method of claim 53, wherein the product saccharide is produced at a concentration of at least about 1 mM.

57. (As filed) The method of claim 53, wherein the cell is permeabilized.

58. (As filed) The method of claim 53, wherein the cell is an intact cell.

59. (Once amended) The method of claim 53, wherein the accessory enzyme for forming a nucleotide sugar comprises an enzyme that is encoded by a heterologous gene.

60. (Once amended) The method of claim 59, wherein the enzyme encoded by the heterologous gene is one or more of:

a GDP-mannose dehydratase, a GDP-4-keto-6-deoxy-D-mannose 3,5-epimerase, and a GDP-4-keto-6-deoxy-L-glucose 4-reductase;

a UDP-galactose 4' epimerase;

a UDP-GalNAc 4' epimerase;

a CMP-sialic acid synthetase;

a pyrophosphorylase selected from the group consisting of a UDP-Glc pyrophosphorylase, a UDP-Gal pyrophosphorylase, a UDP-GalNAc pyrophosphorylase, a GDP-mannose pyrophosphorylase, and a UDP-GlcNAc pyrophosphorylase; a kinase selected from the group consisting of myokinase, pyruvate kinase, acetyl kinase, creatine kinase, UDP-Glc-dehydrogenase; and

pyruvate decarboxylase.

61. (As filed) The method of claim 59, wherein the enzyme for forming a nucleotide sugar and the glycosyltransferase are expressed as a fusion protein.

62. (As filed) The method of claim 61, wherein the fusion protein comprises a CMP-sialic acid synthetase activity and a sialyltransferase activity.

63. (As filed) The method of claim 61, wherein the fusion protein comprises a galactosyltransferase activity and a UDP-Gal 4' epimerase activity.

64. (As filed) The method of claim 61, wherein the fusion protein comprises a GalNAc transferase activity and a UDP-GlcNAc 4' epimerase activity.

65. (As filed) The method of claim 53, wherein the nucleotide sugar is GDP-fucose and the glycosyltransferase is a fucosyltransferase.

66. (As filed) The method of claim 53, wherein the cell forms the nucleotide sugar at an elevated level compared to a wild-type cell.

67. **(As filed)** The method of claim 66, wherein the elevated level of nucleotide sugar results from a deficiency in the ability of the cell to incorporate the nucleotide sugar into a polysaccharide normally produced by the cell.

68. **(As filed)** The method of claim 67, wherein the deficiency is due to a reduced level of a polysaccharide glycosyltransferase activity.

69. **(As filed)** The method of claim 53, wherein the cell/nucleotide sugar are selected from the group consisting of:

Azotobacter vinelandii/GDP-Man;
Pseudomonas sp./UDP-Glc and GDP-Man;
Rhizobium sp./UDP-Glc, UDP-Gal, GDP-Man;
Erwinia sp./UDP-Gal, UDP-Glc;
Escherichia sp./UDP-GlcNAc, UDP-Gal, CMP-NeuAc, GDP-Fuc;
Klebsiella sp./UDP-Gal, UDP-GlcNAc, UDP-Glc, UDP-GlcNAc;
Hansenula jadinii/ GDP-Man, GDP-Fuc;
Candida famata/UDP-Glc, UDP-Gal, UDP-GlcNAc;
Saccharomyces cerevisiae/UDP-Glc, UDP-Gal, GDP-Man, GDP-GlcNAc; and
X. campesti/UDP-Glc, GDP-Man.

70. **(As filed)** The method of claim 53, wherein the cell is *Azotobacter vinelandii*, the nucleotide sugar is GDP-mannose, the acceptor saccharide is lactose, the glycosyltransferase is mannosyl transferase, and the product saccharide is mannosyl lactose.

71. **(As filed)** The method of claim 53, wherein the cell is *E. coli*, the nucleotide sugar is CMP-sialic acid, the acceptor saccharide is lactose, the glycosyltransferase is a sialyltransferase, and the product saccharide is sialyllactose.

72. **(New)** The method of claim 53, wherein the glycosyltransferase consists essentially of a catalytic domain of the glycosyltransferase.